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Urine Analysis

Report on the Workshop Conference of the German Society for Clinical Chemistry and the Society of Nephrology in Würzburg, October 25–26, 1985

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Summary: In an expert workshop conference amongst 45 members of the Society of Nephrology and the German Society for Clinical Chemistry, topical aspects and problems of urine analysis were presented and discussed. The present report summarizes the most significant results, points arising in discussion, and recommendations for test strip analysis, microscopic urine analysis, determination and differentiation of proteinuria and urine enzymology.

Harn-Analytik
Bericht über die Kleinkonferenz der Deutschen Gesellschaft für Klinische Chemie und der Gesellschaft für Nephrologie
Würzburg, 25.–26. Oktober 1985

Zusammenfassung: In einem Expertengespräch mit 45 Teilnehmern aus der Gesellschaft für Nephrologie und der Deutschen Gesellschaft für Klinische Chemie wurden aktuelle Probleme der Harnanalytik vorgetragen und ausführlich diskutiert. Der vorliegende Bericht enthält die wesentlichen Ergebnisse, in der Diskussion geäußerte Meinungen und Empfehlungen zur Analytik mit Teststreifen, zur mikroskopischen Harnanalytik, Eiweißbestimmung und Eiweißdifferenzierung sowie der Enzymanalytik im Harn.

Introduction

Urine analysis is one of the traditional pillars of laboratory diagnostic medicine. It belongs to the basic screening programmes for evaluation of patients of nearly all medical subspecialities. In spite of its importance, and probably due to the rapid development of quantitative and highly differentiated blood tests, the value of qualitative urine analysis is often underestimated *(Scheler)*. In view of an increasing number of patients with chronic renal diseases on

the one hand and the recent development of new techniques on the other hand it seemed useful to define the state of the art in an expert workshop conference between nephrologists and clinical chemists. It was the aim of this meeting to define the actual need from the nephrologist's viewpoint and to discuss possible future strategies in urine analysis considering recent methodological developments. The participants presented their own experiences covering qualitative urine analysis, determination and differentiation of proteinuria, analysis of lithogenic substances and urinary enzymes. Abstracts of contributions were published in 'Nieren- und Hochdruckkrankheiten, Vol. *15*, pp. 205–224, 1986' and in the 'Mitteilungen der Deutschen Gesellschaft für Klinische Chemie, Vol. *17*, pp. 82–89, 116–152, 1986'.

Qualitative Tests

Qualitative urine analysis is mainly used in screening programmes to exclude non-diseased patients. For this purpose qualitative tests with high diagnostic specificity seem sufficient. The simplicity and rapidity of these tests has led to their adaptation for primary diagnosis. In addition these tests can be applied in therapy control in medical practice and self-monitoring *(Scheler)*.

Test strips

The role of test strips and selection of optimal profile for diagnostic purposes in clinical nephrology

Test strips have nearly completely replaced previous chemical urine tests *(Appel)*. The detection limit is adapted to the respective diagnostic needs, aiming high diagnostic specificity to exclude non-diseased patients. Optimal discriminatory function is to be expected if the maximal analytical sensitivity (concentration giving a positive answer in 10% of patients) is near the practical sensitivity (concentration where 90% of urines give a positive result) *(Kutter)*. This aim seems to have been achieved for the detection of erythrocytes, albumin and glucose, but improvement is still desirable in the detection of leukocytes. The optimal screening profile from the nephrological viewpoint is: pH, protein, leukocytes, erythrocytes. In view of possible future developments it would be desirable to have an additional test for the relative density or osmolality to improve interpretation. This seems especially useful when test strips are read by mechanised test strip photometers. Other test fields presently included in test strip screening profiles, like nitrite, urobilinogen, bilirubin and ketone bodies were said to be less useful in screening programmes. This is partly due to the low analytical specificity. Confusion increases with the number of test fields, simultaneously decreasing the correctness of the reading with time. In addition, the desire to use test strips in 10 ml tubes supports the need for a reduction of test strip length.

Centralization of test strip analysis?

Qualitative testing of urine samples is of diagnostic value only if proper sampling and pre-analytical handling of samples is assured *(Thomas)*. The discussion of who should perform test strip analysis was controversial: on the one hand, performance of the analysis close to the patient offers some advantages (less time consuming, pre-selection of non-diseased, better control of pre-analytical steps); it seems, however, only to apply when carried out by a trained person. When performed by untrained or uncontrolled staff, results become much less reliable. Besides performance problems, non-observation of expiry dates and improper storage of test strips can lead to misinterpretations *(Renner)*. Centralized performance of test strip analysis, on the other hand, offers the possibility of automation. The advantages of automated analysis with the use of reflectance photometers (constant timing, optical compensation) is presently still hampered by some dis-advantages: Visible colour interferences cannot always be compensated for in detecting haemoglobin, and single spots cannot be differentiated from homogeneous changes in colour *(Haeckel)*. Even the most recently developed analysers, Urotron, (Boehringer-Mannheim) Rapimat II, (Behring), Clinitec (Bayer-Diagnostic), do not perform a test strip analysis more rapidly than visual reading. Evaluation of test strip analysers according to the ECCLS protocol raised several problems: reference methods were missing for some parameters, and statistical models were not available for qualitative discontinuous scales, which are presently used in spite of continuous signals (1). In future developments, it seems possible that quantitative results will be achieved. In spite of the limited diagnostic value of test strip analysis this seemed, however, questionable, if results are not referred to creatinine, osmolality or time *(Breuer, Haeckel)*.

Accordingly, external quality control gives random results if concentrations are around the border between two steps. Experimental collaborative, interlaboratory trials with pathological urines, however, resulted in 71–92% accurate results *(Appel)*.

Test strip preselection of non-diseased?

Test-strips are suggested for preselection of non-diseased patients, whose samples may not need microscopic examination. This was said not to be valid for all cases. The participants agreed that the sensitivity of the blood detection test strip was sensitive enough to exclude clinically significant haematuria. The sensitivity of the leukocyte test strip (based on esterase activity measurement) seems, however, insufficient to exclude leukocyturia. Besides the number of leukocytes, urine osmolality, time of storage and age of granulocytes seem to influence the result *(Guder)*. Since granulocytes from the outer genitals often contaminate urine this may be advantageous. After freezing and thawing of urines or after prolonged storage, a higher number of positive results is obtained (2).

Likewise a negative result from a protein test strip does not exclude a clinically relevant proteinuria. This is mainly caused by the higher sensitivity of the testfield for albumin. Systematic re-evaluation of tubular proteinurias led to the conclusion that most urines with increased concentration of low molecular weight proteins give a negative test strip result (tab. 1, *Alt*). This means that test strips cannot be used to exclude tubular proteinurias (3). Likewise *Bence-Jones* proteins are not always detected. In addition, microalbuminuria of diabetics cannot be detected by test strips (20–200 mg/l). Considering that urine samples are not always representative, test strip analysis in fresh urine samples seems only useful for screening purposes (4). In comparison with the microscopic examination, a diagnostic sensitivity of 96.7%, a diagnostic specificity of 86.3%, a false positive rate of 5% and a false negative rate of 0.5% was found ((4), *Binswanger*). Nephrological diagnosis requires additional microscopic and biochemical examination.

Tab. 1. Comparison of test strips and disc electrophoresis in evaluating proteinuria.
Urines were collected from people aged over 70 years exhibiting a high proportion of changed urine protein patterns. Numbers in brackets give the percentages of the collective showing the same pattern. Tubular proteinuria was diagnosed by micrograadient electrophoresis, exhibiting relative and absolute increases in low molecular weight proteins (*Alt*).

Test strip result	Electrophoretic pattern of protein			
	normal	tubular	glomerular	mixed pattern
−, (+)	82 (60)	15 (50)	0 (0)	5 (23)
+	52 (38)	13 (43)	8 (67)	9 (41)
++	2 (2)	2 (7)	3 (25)	7 (32)
+++	0 (0)	0 (0)	1 (8)	1 (4)
Sum	136 (100)	30 (100)	12 (100)	22 (100)

Cells

Erythrocytes, haemoglobin

Any positive result from an erythrocyte test strip should be followed by microscopical examination to clarify the type of haematuria. The aim is to differentiate pre-glomerular (intravasal haemolysis) from glomerular (glomerular nephritis) and post-glomerular (kidney stones, tumours, cystitis) haematuria *(Renner)*.

This precludes clear definition of the type of urine examined (spontaneous, middle portion, catheter or suprapubic punction urine). Macroscopic inspection of urine before and after centrifugation can help to differentiate between haematuria and haemoglobinuria. Lacquer-coloured transparent urine is typical for haemoglobinuria whereas opaque transparent urine points to corpuscular elements *(Binswanger)*. Microscopic examination should be performed not later than 2 hours after sampling.

Two different modifications can help to differentiate haematuria: Preparation of a *concentrated urine sediment* from 40 ml urine increases the incidence of erythrocyte-containing casts in glomerular haematuria from 24 to 80% positive results *(Walb)*. Examination of *erythrocyte morphology* is performed with a phase-contrast microscope: 20 ml urine are centrifuged at 2000 min^{-1} for 3 minutes, 9.5 ml of the supernatant discarded and the remainder resuspended; 20 µl of the suspension are examined at 400-fold magnification by phase-contrast. After examining at least 100 erythrocytes or 10–20 visual fields, typically deformed erythrocytes with extrusions (so-called akanthocytes) are counted (fig. 1, *Köhler*, (5)). If the number of typically deformed erythrocytes is above 10%, glomerular haematuria can be assumed with 99% specificity. However, below this number

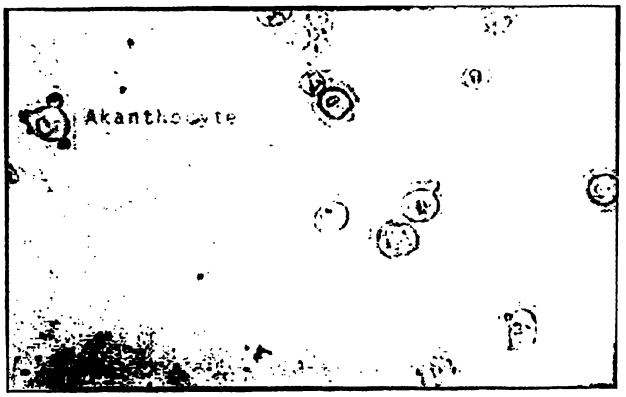

Fig. 1. Dysmorphic erythrocytes in urine. Phase contrast microscopy *(Koehler)*.

50% of haematuria cases are still of glomerular origin. Even the so-called physiological haematuria is of the glomerular type. Therefore clinically significant glomerular haematuria can only be considered if erythrocyte numbers are above 8/µl. However, classification may be difficult in cases of micro-haematuria without clinical symptoms, especially if only empty erythrocyte ghosts are visible *(Renner)*. Careful interpretation is therefore suggested at borderline haematuria. The dysmorphy of erythrocytes seems to be caused predominantly by the distal nephron passage of cells, and to a lesser degree by passage through glomerular basement membrane *(Heidland)*. The proportion of dysmorphic erythrocytes is decreased after furosemide and water diuresis (fig. 2, (6)). This assumption is supported by the observation that typically changed erythrocytes and erythrocyte casts are likewise observed in renal tumours.

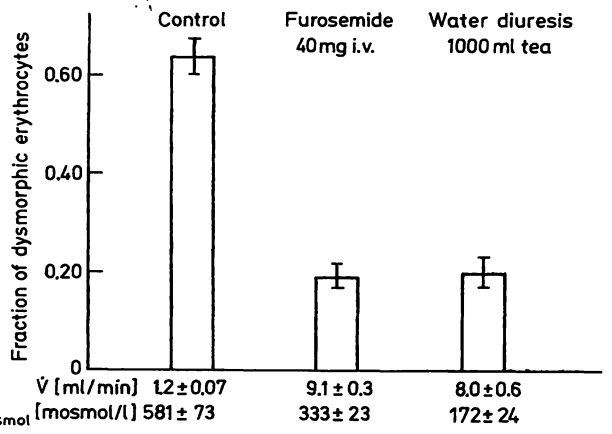

Fig. 2. Effect of furosemide and water diuresis on the fraction of dysmorphic erythrocytes in urine (*Heidland*, (6)).

In summary, differentiation of erythrocyte morphology seems to be useful only if a positive test-strip result is obtained at a specific weight above 1015, contamination being excluded (middle portion) *(Walb)*.

Leukocytes, epithelia and casts

Examinations of urine sediment needs time and experience *(Renner)*. Examination of the unstained urine seems to be sufficient for detection of infectious granulocyturia. In other cases differentiation of leukocytes in stained smears offers a valuable additional possibility *(Sandoz, Winkelmann)*. Differentiation of 100 nucleated cells on prefabricated glass slides (Test Simplets®) allows the separation of polymorphonuclear cells from lymphocytoid cells. The presence of more than 20% lymphocytoid cells with less than 55% polymorphonuclear cells indicates an interstitial rejection crisis after kidney transplantation, with a diagnostic sensitivity of 91% (7). In addition, the number of lymphocytes increases in acute renal failure and in cytomegaly virus infections *(Sandoz)*. Examination of the second urine of the day, by separation with the cytocentrifuge and *Papanicolaou* staining, shows that 100% of cases with the onset of a rejection crisis exhibit tubular epithelia, 95% exhibit casts, and 66% exhibit a so-called dirty cell picture, apatite crystals (hourglass form) and mitoses *(Winkelmann)*.

Increased finding of plaster epithelia indicates improper urine sampling *(Binswanger)*. Giant mitochondria in tubular epithelia point to cyclosporin toxicity, while cellular virus particles signal cytomegaly virus infections *(Winkelmann)*.

The discussion resulted in further suggestions to improve the diagnostic value of urine sediment analysis. Standardization of urine volume, the suspension volume, counting chamber and magnification markedly increases the reproducibility of sediment analysis *(Lison)*. For future developments the use of cell sorters seems useful in analysing urine cells *(Thomas)*. This would allow the analysis of cells covered with *Tamm-Horsfall* protein *(Walb)*, thus indicating their tubular origin.

Proteinuria

Analysis of total protein excretion

Test strip positive proteinuria should be quantified. In addition, the pattern of proteinuria should be examined. The aim of analytical procedures is to differentiate between glomerular and tubular proteinuria, and separate selective from non-selective glomerular types *(Renner)*. Only 10% of the normal urinary proteins are derived from plasma. The glomerular filtration rate of each protein is characterized by the sieving coefficient, which is 0.3×10^{-3} for albumin. This coefficient depends on the molecular radius, electric charge and isoelectric point of the protein *(Schurek)*.

Any quantifying method should be performed in a 24-hour urine. From a large variety of methods the biuret procedure and dye-binding methods have found widest acceptance. In addition, light scattering methods (8) and the *Lowry* procedure were discussed. In selecting the method, the different chemical principles, specificities and normal values have to be considered. The method of *Lowry*, which responds mainly to tyrosine, is not useful for urine because of its lack of specificity. The widespread biuret proce-

dure, on the other hand, which measures peptide bonds, is only applicable after precipitation and washing of proteins. Perchloric acid is the preferred precipitant because it also precipitates polypeptides *(Thomas)*. Precipitation should be performed over 10 minutes in ice, and the precipitate should be separated at a centrifugal force above 2000 *g*. For normal and slightly elevated concentrations (negative test strips) 10 ml urine should be used. Precipitate should be re-dissolved in 1 ml reagent.

The use of dye-binding procedures with Coomassie-brillant-blue or Ponceau-S allows quantitation of protein in 20 µl urine. Limited linearity can be overcome by bichromatic measurement at 595/465 nm up to a concentration of 4 g/l *(Peheim)*. In spite of good correlation of these procedures with the biuret method and excellent intra-assay and inter-assay precisions (below 3% CV) dye-binding procedures have diagnostic drawbacks: In contrast to the biuret-procedure, values for albumin are decreased in diabetic urines, *Bence-Jones* proteinuria is less often detected, and interference factors are encountered more often because proteins are not precipitated before measurement *(Thomas)*. Thus contrast media, which precipitate at acid pH, can interfere with all binding procedures *(Kutter)*, dextran interferes with the Ponceau-S method above 2 g/l, but not with the Coomassie-blue procedure *(Appel)*. Alternatively, the *nephelometric procedure* after addition of trichloroacetic acid can be performed with a rate nephelometer or fluorimeter (8). It detects albumin and IgG with the same light intensity and gives sufficiently good recoveries even in the normal range *(Kirchherr)*. The following drugs had no influence: penicillin, amoxicillin, gentamycin, furosemide, dihydralazin. Contrast media (adipiodon/meglumin, Biligrafin®) interfere because they precipitate in acid media.

Independently of method-related differences in the determination of total protein in urine, standardization is also hampered by the lack of an international standard. Depending on the preparation procedure, different batches of albumin can react with different intensity. In addition, even the purest albumin has to be dehydrated over phosphorus pentoxide *(Pfleiderer)*. Besides purified albumin, the American urine protein standard NBS, control serum and serum protein standards are used. When standardized with highly purified albumin, normal values range from 40 to 150 mg/24 hours with the biuret procedure, 25–75 mg/24 hours for the nephelometric procedure, and 40–100 mg/24 hours for the dye-binding methods. Drugs interfere with all methods mentioned. Observations on drug interference are collected and published by the working group on drug interferences of the German Society of Clinical Chemistry, compared with Swedish and British computer programs (9), and if not known included in the programmes *(Breuer)*.

The discussion culminated in the conclusion that whatever you do with standards in measuring urinary protein the result will be wrong *(Thomas)*, leading to the suggestion of clinicians that total protein be replaced by specific methods to measure defined single proteins *(Renner)*.

Differentiation of proteinuria

Electrophoretic procedures

Examination of urine protein composition can be performed with a wide variety of recently developed methods. Qualitative one-dimensional polyacrylamide gel electrophoresis differentiates all types of proteinuria. Quantitative determination of single proteins with defined molecular weight, two-dimensional electrophoresis and the use of monoclonal antibodies against defined glomerular, proximal or distal tubular proteins has widened the dimension of analytical possibilities. Clinical significance, however, has still to be evaluated. On the basis of the reported experiences, it is to be hoped that a widening of the analytical spectrum will improve differential diagnosis and increase the awareness of renal functional defects. By combining polyacrylamide gel electrophoresis with quantitative determination of albumin *Renner* was able to show that even with normal albuminuria (below 16 mg/24 hours) the protein pattern was normal only in 73% of cases, whereas 27% had a pathological protein pattern (tab. 2 a). This study confirms that neither negative test strip results nor quantification of albumin can exclude pathological proteinuria. This is mainly due to the large number of tubular proteinurias, which account for 23% of urine samples in a nephrological clinic. In 80% of cases with albumin values increased up to 80 mg/24 hours, the urines exhibited a pathological pattern (tab. 2 b). Albumin values measured either by kinetic nephelometry or by enzyme-immunological methods correlate well with results obtained by radioimmunoassay. The discussion led to the conclusion that pathological urine protein patterns can be excluded or confirmed only by analysing the type of proteinuria.

Sensitivity of detection can be increased with the silver staining procedure which allows detection of 0.3 mg/l protein. β_2-Microglobulin, haemoglobin monomers, lysozyme, and retinol-binding globulin can be characterized by their black-brownish colour, free light chains appear light-brown, α_1-microglobulin

Tab. 2. Protein pattern in patients with microhaematuria (N = 813) with normal (a) and slightly elevated (b) albumin excretion in 24 hour urine (*Renner*).

a) Physiological proteinuria. Albumin below 16 mg/24 h.

Pattern			
physiological	351 =	73 %	73%
glomerular	10 =	2 %	27%
tubular	91 =	19,2%	
vascular	10 =	2 %	
mixed proteinuria	9 =	1,8%	
plasma contamination	1 =	0,2%	
unclear	9 =	1,8%	
Sum	481	100 %	

b) Minimal proteinuria. Albumin 16–80 mg/24 h.

Pattern			
physiological	66 =	20 %	20%
glomerular	99 =	30 %	80%
tubular	81 =	24 %	
vascular	35 =	11 %	
mixed proteinuria	30 =	9 %	
plasma contamination	10 =	2,9%	
unclear	11 =	3,1%	
Sum	332	100 %	

grey-brown. This observation makes interpretation of stained protein patterns easier (*Schiwara*, (10)). To avoid artefacts due to long incubation of urine in the bladder, urines of high osmolality should be excluded prior to the analysis of protein patterns. Filtration or concentration of urine should likewise be avoided since atypical bands can be lost by absorption *(Scherberich)*. Separation can be performed in micro slab gel-electrophoresis ((11), *Weber*), in ultrathin layer SDS-electrophoresis *(Boesken, Schiwara)* or in microgradient electrophoresis columns which allow quantification ((3), *Alt*). Even HPLC-techniques have been used to differentiate urinary proteins (12). This technique offers the possibility of quantifying fractions, but separation is not yet satisfactory *(Wisser)*.

Interpretation of electrophoretically separated protein patterns requires experience, since the pattern can be changed by extrarenal factors (i. e. haemoglobin), contamination and artefacts. Selective glomerular, glomerulo-tubular and tubular proteinurias can follow each other in the course of therapy in diabetics (13). When analysing the urine of 126 pregnant women, tubular and glomerular proteinuria were detected 24 times each *(Lison)*. Fifteen pregnant women exhibiting glomerular proteinuria during the first trimenon, developed hypertension, while an additional 5 cases developed hypertension during the following gravidity. Glomerular proteinuria appeared 6 weeks before the first clinical symptoms of eclampsia. Consequently electrophoretic protein analysis should be incorporated into pregnancy care programmes.

Quantification of single proteins

As an alternative to the electrophoretic separation, a simultaneous quantification of albumin, a microprotein and transferrin was proposed. α_1-Microglobulin seems to be superior to the previously measured β_2-microglobulin, since its excretion rate is relatively constant (3.5–8 mg/l), given its stability in acid urines ((14), *Weber*). Increased α_1-microglobulin concentrations in urine, however, only indicate tubular functional impairment if the glomerular filtration rate is in the normal range. Immunofixation of defined proteins after gel electrophoresis allows semiquantitative evaluations *(Bauer)*. The clinical information, however, is not improved by this procedure. The same still holds for two-dimensional electrophoresis which allows separation of up to 500 single protein spots. Only after identification of defined proteins from different segments of the renal tubule can an increase in diagnostic efficiency be expected *(Pfleiderer)*. This has already been achieved by using monoclonal antibodies for the determination of defined segment proteins *(Müller)*. Thus cis-platinum treatment leads to the excretion of a distal tubular antigen in urine *(Falkenberg)*. Proximal and distal antigens in urine seem to be an early indication of a rejection crisis: in these studies, an antigen from the antiluminal membrane of the distal tubule was identified in urine for the first time. The use of antibodies against membrane antigens not only allows detection of proteins but also of antigen-labeled cells *(Scherberich)*.

The well known tubular glycoprotein, the so-called *Tamm-Horsfall* glycoprotein, represents the main fraction of normal urinary proteins (25–40 mg/24 h). It contains 28% carbohydrate, and exhibits an isoelectric point of 3.5. In renal diseases that led to a decrease in the number of tubules, excretion of this protein is decreased accordingly. This is of limited diagnostic significance, but the measurement of this glycoprotein in serum seems to be an interesting indicator of intrarenal reflux *(Rambausek)*. In diabetics the carbohydrate composition and isoelectric point of *Tamm-Horsfall* glycoprotein are changed. This is probably responsible for the decreased solubility of the protein in diabetes, and the increased susceptibility of diabetics to acute renal failure after the administration of X-ray contrast media *(Rambausek)*.

Quantification of urinary albumin under basal and exercise conditions was used as a provocation test. Whereas ergometry does not normally lead to an increase in albuminuria, diabetics in the initial stages of microangiopathy exhibit albuminuria under these conditions.

In conclusion, the observations encourage the performance of differentiated analysis of proteins in urine as early as the first diagnostic step. Tubular proteinuria can only be detected by measuring single microglobulins and/or performing microelectrophoresis. To differentiate a test strip-positive proteinuria, at least three different proteins with different molecular weights have to be measured (α_1-microglobulin, albumin, transferrin or IgG). Alternatively, SDS-polyacrylamide gel electrophoresis can be used, but this only qualitatively differentiates selective and unselective glomerular and tubular proteinuria.

Urine Analysis in Kidney Stone Formers

In a session that was apparently too short, the participants discussed a programme designed for monitoring kidney stone formers *(Tschöpe, Hesse)*. Three mechanisms have been identified in kidney stone formation and its prophylaxis:

1. Retention time of microparticles
2. Inhibitor potential of urine in the prevention of crystallization
3. Oversaturation of stone forming ions.

Particle retention time depends on the tendency of anatomical structures to retain preformed microcrystals. With increasing retention time the probability increases that crystals will adhere and develop into macroscopic stones. At present it is impossible to measure the time difference between formation of microcrystals and their elimination.

Inhibitory potential, on the other hand, is defined as the sum of chemically defined substances which either inhibit nucleation, aggregation or the speed of development of crystals. The inhibitory potency of a given substance depends on the matrix and is different for each kind of stone. Thus citrate, pyrophosphate, chondroitin-sulphate and ribonucleic acids are potent inhibitors of calcium oxalate stone formation. Citrate and pyrophosphate are directly relevant for prophylactic and therapeutic management of stone formers. At present standardized techniques to measure the inhibitory activity of an individual urine is lacking, at least for routine purposes. A saturation status of lithogenic ions in urine, on the other hand, has attracted attention, since results can directly be applied in therapeutic decisions. Stone formers can best be separated from non-stone formers, if oversaturation and inhibitor potential are mathematically related in diagrams *(Tschöpe)*. This explains why patients with high degrees of oversaturation do not form stones, if the inhibitory potential is likewise high. In contrast, patients with a low inhibitory potential can form stones even at normal calcium and oxalate excretion rates. From these considerations, it can be concluded that the presently used diagnostic programme, the measurement of electrolytes in urine, is only of limited diagnostic usefullness with regard to the pathogenesis of kidney stones. It is, however, the basis of any systematic nephrolithiasis related laboratory, since a series of primary causes (intestinal hyperabsorption of calcium, primary hyperparathyreoidism, renal tubular acidosis, excessive sodium and calcium uptake, hypervitaminosis D, sarcoidosis, mobilisation of endogenous minerals (resorptive hypercalciuria), primary oxalosis type I and II, absorptive hyperoxaluria) can be diagnosed or excluded with this programme.

For this purpose it is important to have reference values. These criteria are, however, hardly to fulfill in a non-selected population. They are lacking for the important inhibitors, citrate, pyrophosphate and glycosaminoglycans. This is partially due to the unsufficient stabilisation of urines and low number of cases. Table 3 summarizes the 5—95 percentiles of calcium and sodium excretion rates and the calcium/creatinine ratio of non-diseased people in a Heidelberg normal population between 20 and 40 years *(Tschöpe)*. Table 4 represents the parameters used in the kidney stone research laboratory, Bonn *(Hesse)*. It may be pointed out that the critical limits for kidney stone formers do not coincide with the upper normal limit.

Tab. 3. 5—95 Percentiles of calcium and sodium excretion rates (mmol/24 h) as well as calcium/creatinine ratios (mol/mol) of healthy normal people from the Heidelberg region (238 men, 145 women, age 20—40 years, with no dietetic prescriptions) (15).

	Men	Women
Calcium (mmol/24 h)	1.84— 10.9	1.65— 8.37
Calcium/creatinine (mol/mol)	0.12— 0.6	6.15— 0.74
Urinary sodium (mmol/24 h)	81 —332	63 —252

When hypercalciuria is found (above 10.9 mmol/24 h in males, 8.4 mmol/24 h in females) it should be differentiated as absorptive (intestinal absorption) or resorptive hypercalciuria. This can be performed with the sodium cellulose phosphate test: it is done by measuring the calcium/creatinine ratio in the morning urine before and after inhibition of intestinal absorption of calcium with the aid of sodium cellulose phosphate (15).

Any laboratory analysis of stones or urine in stone formers should be done either to elucidate the pathogenesis of stone formation or to prevent recidives. In analysing stones, the chemical analysis no longer meets the demands of the clinical chemist since major errors have been observed in collaborative interlaboratory trials. Infrared spectroscopy and X-ray diffraction analysis are equally potent in analysing the crystal structure of kidney stones *(Hesse)*. In addition, the following minimal programme should be performed using *metaphylaxis of recurrent stone formation:* calcium, uric acid, creatinine in serum and urine, urinary sediment, crystals, bacteriology, test strip and specific weight. Whenever calcium is involved in stone formation, hyperparathyreoidism has to be excluded. A future standard programme may include the determination of oxalate, calcium, uric acid, phosphate, citrate, magnesium, sulphate, pyrophosphate, glycosaminoglycans, sodium, potassium and creatinine (tab. 4) (16).

This programme was not accepted unequivocally. Problems have already been experienced in collecting the urine. Acidification leads to precipitation of uric acid and citrate. Therefore collection after addition of thymol is suggested. Urine must be carefully mixed before samples are separated. This treatment, however, does not prevent in vitro oxalate formation from ascorbic acid (17). A prerequisite for interpretation of results from this standard programme is the standardization of meals before urine collection. The multiplicity of influence factors makes such a large programme meaningful only if there is optimal cooperation between the patient, the therapist and the analyst.

Urinary Enzymes

Urinary enzymes, like other proteins, can be derived from pre-renal, renal and post-renal sources. Activity of plasma enzymes in urine is not only determined by their molecular weight, but likewise by the degree of renal processing (reabsorption, degradation, inactivation) as well as their stability under physiological conditions (37 °C over 1–8 hours at pH 5–9). Thus pancreatic lipase and amylase are filtered at a similar rate. However only amylase is found in urine in appreciable activities, whereas lipase is inactivated during tubular passage (*Mályusz*, (18)). The selection of a urinary enzyme for diagnostic purposes must therefore be closely linked to knowledge of the sources and mechanisms of release of the enzyme. Stability under physiological conditions and insensitivity to the inhibitory potential of normal urine constituents is a prerequisite. By appropriate sample preparation, interfering differences in pH, inhibitors and activators as well as urochromes and drugs can be eliminated *(Maruhn)*. Gel chromatography is sug-

Tab. 4. Reference ranges and risk limits for urolithiasis (24 h urine) (*Hesse*).

Parameter	SI-units		Conventional units		Risk limits in urolithiasis
Specific weight	1.005– 1.030	kg/l	–		> 1.015 g/cm³
pH	5.8 – 6.8		–		< 5.5; >7.0
Calcium	0.25 – 7.50	mmol	0.5– 15.0 (10–300 mg)	mval	> 5 mmol
Magnesium	1.50 – 7.50	mmol	3.0– 15.0 (40–200 mg)	mval	< 3 mmol
Inorganic phosphorus	16 –48	mmol	496 –1487	mg	>35 mmol
Uric acid	2.38 – 4.46	mmol	400 – 770	mg	> 3 mmol
Citric acid	2.08 – 4.16	mmol	400 – 800	mg	< 2 mmol
Oxalic acid	<0.50	mmol	<45	mg	> 0.5 mmol
Cystine	<0.333	mmol	<80	mg	> 0.8 mmol
Sodium	150 –220	mmol	150 –220	mval	–
Potassium	30 – 90	mmol	30 – 90	mval	–
Ammonium	30 – 50	mmol	510 –850	mg	–
Chloride	170 –210	mmol	170 –210	mval	–
Sulphate	15 – 25	mmol	480 –800	mg	–
Creatinine	11.5– 15	mmol	1.3– 1.7	g	–

gested as the superior procedure (19). This eliminates urea, salicylic acid and mitomycin which inhibit N-acetylglucosaminidase. Indomethacin, puromycin and isoxepac, on the other hand, have been found to inhibit alanine aminopeptidase (*Maruhn*, (19)). The degree of inhibition depends not only on the concentration of the inhibitor, but also on the substrate used and the dilution of sample in the assay. Alanine aminopeptidase (using alanine *p*-nitroanilide as substrate) is inhibited by amino acids and ammonia *(Mattenheimer)*. About 50% of the inhibitory potential in normal urines is due to histidine, phenylalanine and ammonia. Other amino acids are less potent, either because they have a high inhibitory constant, or because their concentration in urine is low. The inhibition by substrate excess observed in gel filtrates is counteracted by amino acids and ammonia. Kinetic experiments using increasing inhibitor concentrations indicate the existence of two isoenzymes, of which one is inhibited non-competitively, the other competitively. Whether this is attributable to the soluble or to the membrane-bound enzyme fraction has not yet been clarified. Using leucine *p*-nitroanilide or leucinamide as substrate, inhibition by amino acids is competitive (*Mattenheimer*, (29)). The existence of different aminopeptidases is supported by the analysis of kidney homogenates. Here a superficially attached brush-border enzyme can be differentiated from a cytosolic alanine amide splitting activity *(Scherberich)*.

Agreement was reached on the *optimal specimen for urine enzyme analysis*. The second urine in the morning, whose time of formation and volume is known, was said to be optimal. Thus results can be related to creatinine (at normal serum creatinine) or time. Activity changes have to be differentiated into those related to volume, time, or creatinine, and those related to changes in the absolute excretion rate. In animal experiments large differences between different breeds of the same species have to be considered *(Simane)*. In addition, urine enzyme activities are changed by hormonal (thyroid hormones), dietetic and circadian influences *(Burchardt)*. In interpreting urine enzyme activities, a classification into *alteration, lesion* and *necrosis* was suggested *(Mondorf)*. By this definition alteration leads to an increase in enzyme content of the kidney in parallel to the excretion (alanine aminopeptidase, γ-glutamyltransferase). In alterations, no changes in tubular function can be observed. Lesion on the other hand is paralleled by changes in proximal tubular reabsorptive function, measured either by detection of renal glucosuria or microglobulinuria. Brush border enzymes are often excreted in aggregates under these conditions. Only in cell necrosis, however, are parts of the cytoskeleton, intracellular enzymes and distal nephron structures recovered from the urine (*Mondorf*, (21)). In addition, the time sequence of enzymuria has to differentiate rapid effects from cumulative actions and chronic effects. In animal experiments it was found that even the transport of animals, and other stress factors lead to an increased excretion of proteins and enzymes in urine *(Alt, Boesken, Maruhn)*. This led to the conclusion that only continuous determination of urine enzyme activity allows an interpretation of hyperenzymuria. Experimental and clinical observations supported this suggestion: After injecting rats with folic acid (250–375 mg/kg), lysosomal and brush border enzymes decreased with a simultaneous increase of cytosolic enzymes (lactate dehydrogenase) ((22), *Maruhn*). An increase in urine enzyme activity over three consecutive days indicated higher risk of acute renal failure on the following days under treatment with cytostatic drugs *(Mondorf)*. Single determinations did not permit this prediction. This was confirmed in studies on urinary enzymes after renal transplantation. By daily measurement of ligandin (gluthatione-S-transferase), pyruvate kinase, fructose-1,6-bisphosphatase and N-acetylglucosaminidase, rejection crises were predicted with a specificity of 97%, if absolute activity and trends over time were combined to calculate probability (*Kotanko*, (23)). In 59 patients with kidney transplantation, 21 had histologically verified rejections, 9 of these being predicted two days before, 7 on the day of clinical diagnosis, and 1 either one or two days after the crisis. Cyclosporin A treatment suppresses clinical signs of rejection. The nephrotoxic effect of this drug, however, likewise reduces the diagnostic value of urinary enzyme measurement *(Scherberich)*. Addition of further parameters does not improve the gain in information *(Wisser)*.

Urine enzyme analysis can also be used to detect nephrotoxic actions of drugs and to monitor diabetic nephropathy. Thus alanine aminopeptidase excretion decreases under graded exercise in normals, whereas it increases in diabetics *(Schoeppe)*. The change in enzyme excretion after exercise did, however, not correlate to the degree of microangiopathy *(Guder)*.

Automation of methodology seems to be a prerequisite for the use of urine enzyme analysis in screening profiles. This is now possible using a new substrate for N-acetylglucosaminidase, which allows measurement at saturation and is independent of endogenous inhibitors *(Guder)*. Alanine aminopeptidase can likewise be measured automatically after gel filtration *(Wisser, Guder)*.

Urinary kallikrein plays a specific role among urinary enzymes since it stems from the connecting tubule, the final part of the distal convoluted tubule *(Guder)*. Its activity measurement in urine and interpretation of the result are complicated by a large variety of influence factors, the presence of endogenous inhibitors, as well as method-dependent differences *(Guder, Bönner, Hörl)*.

In summary, the impression remained that interpretation of urine enzyme activity seems much more difficult than that of plasma or serum enzymes. This is partially due to the fact that urinary enzymes are released into an open system which does not allow the diagnostically important accumulation of these biochemical markers.

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